Pergamon

Pharmacology Biochemistry and Behavior, Vol. 49, No. 1, pp. 47–56, 1994

0091-3057/94 $6.00 + .00

0091-3057(94)E0087-X

Evidence for Central Benzodiazepine Receptor Heterogeneity From Behavior Tests

M. F. DAVIES,*[1] E. S. ONAIVI,† S.-W. CHEN,* P. A. MAGUIRE,*
N. F. TSAI* AND G. H. LOEW*

*Molecular Research Institute, 845 Page Mill Road, Palo Alto, CA 94304
†Meharry Medical College, Department of Pharmacology, Nashville, TN 37208

Received 2 October 1993

DAVIES, M. F., E. S. ONAIVI, S.-W. CHEN, P. A. MAGUIRE, N. F. TSAI AND G. H. LOEW. *Evidence for central benzodiazepine receptor heterogeneity from behavior tests.* PHARMACOL BIOCHEM BEHAV **49**(1) 47–56, 1994. – To explore behavioral selectivity as a consequence of multiple receptor subtypes, four benzodiazepine receptor ligands, flunitrazepam, CGS 9896, zolpidem, and AHR 11797, were tested at five in vivo endpoints: anticonvulsant action, anxiolysis/anxiogenesis as determined in the plus-maze test, locomotor activity, changes in food consumption, and hypothermia. All compounds produced hypothermia. In the plus-maze test, flunitrazepam, CGS 9896, and a low dose of zolpidem (0.05 mg/kg) increased the time spent in the open arms, although AHR 11797 and higher doses of zolpidem decreased time spent in the open arms. Flunitrazepam and zolpidem greatly reduced, CGS 9896 slightly reduced, and AHR 11797 did not affect locomotor activity. Flunitrazepam and CGS 9896 increased food consumption, but AHR 11797 and zolpidem had no effect. Only flunitrazepam fully protected the animals from pentylenetetrazol-induced seizures. The qualitative differences in the effects of these compounds observed are difficult to explain by activation of a single benzodiazepine receptor subtype. As Ro15-1788 antagonized all the observed effects, these compounds act through multiple central benzodiazepine receptors.

Central benzodiazepine receptor heterogeneity Flunitrazepam CGS 9896 AHR 11797 Zolpidem Ro15-1788 Plus-maze test Anticonvulsant test Hyperphagia Locomotion in open field Hypothermia Central benzodiazepine binding

THE MULTIPLE behavioral actions of benzodiazepine (BDZ) receptor agonists make them clinically useful anxiolytics, anticonvulsants, muscle relaxants, and hypnotic agents. It is also known that they can affect food and water consumption (6) and reduce body temperature (18). It was originally assumed that all BDZ receptor agonists had similar pharmacological profiles, acting through a single BDZ receptor to produce every observed behavioral action, although not necessarily in the same dose range (17). A direct consequence of this hypothesis is that, if the effects of a set of compounds at diverse in vivo endpoints were to be measured under identical conditions, the rank order of activity of these compounds at each endpoint would be the same, but the dose range required for each activity could be different.

The concept that all BDZ effects are mediated by a single BDZ receptor has been challenged by recent behavioral, receptor binding and molecular biology studies. For example, in behavioral studies, the traditional 1,4 benzodiazepines have anticonvulsant and anxiolytic effects at lower doses than are required to produce sedation (17), whereas the novel benzodiazepine compound zolpidem produces sedative effects at doses much lower than those required to cause anticonvulsant or anxiolytic effects (14). Both binding studies and the techniques of molecular biology provide direct evidence for multiple BDZ receptor subtypes. Early receptor binding studies had detected two central BDZ receptors (20,35), and on this basis these subtypes were called Type I and II. Our laboratory has found evidence for more than two pharmacological relevant subtypes of BDZ receptors in the spinal cord (22), and using a novel data analysis technique – Fourier-derived affinity spectrum analysis – we have recently resolved at least three central benzodiazepine binding sites in the spinal cord (submitted). Evidence for multiple $GABA_A$/BDZ receptor subtypes has also been provided by molecular biology techniques that have characterized many $GABA_A$ receptor subunit variants (21). Expression of combinations of these subunits in various transfected cell systems produce BDZ receptors that have different ligand affinities (29) and varying ability to be activated by

[1] To whom requests for reprints should be addressed.

BDZ receptor ligands (30). For behavioral selectivity to occur, the BDZ receptor subtypes within the CNS must be localized to specific regions, and it is known that GABA receptor subunit variants are not distributed evenly throughout the CNS (37).

The goal of this study was to use internally consistent endpoints to continue to explore the possibility that a single compound could behave in a qualitatively different manner when tested using different in vivo endpoints. Use of standardized procedures more readily permit the comparisons both between drugs and between endpoints allowing the determination of the drugs' qualitative effectiveness and rank order of activity in each test. To this end, we have examined the action of four structurally diverse BDZ receptor ligands in five in vivo endpoints known to be sensitive to BDZ receptor ligands: anticonvulsant action against pentylenetetrazol (PTZ), anxiolysis/anxiogenesis as determined in the elevated plus-maze test, locomotor activity as an indication of sedation, changes in food consumption (6), and change in body temperature (18).

The test compounds were chosen based on some prior evidence for selective behavioral actions that could not be explained by activation of a single BDZ receptor. Flunitrazepam (Fig. 1) was included among the four compounds as an example of a classical 1,4 benzodiazepine that is thought to have full agonist activity (5). Zolpidem, an imidazopyridine (Fig. 1), was reported to be particularly sedative at doses lower than those required to produce anticonflict action (14,23), but lacked effect on feeding behavior (38), and had relatively weak anticonvulsant activity (14). It represented a compound in which anxiolysis and anticonvulsant action did not seem to be strongly linked. CGS 9896, a pyrazoloquinoline (Fig. 1), produced very little sedation (4,32), was reported to be a selective anxiolytic compound (3,26,31) that also increased mouse exploratory behavior (4,31), and blocked the pentylenetetrazol discriminative cue (4). The anxiolytic action of CGS 9896 was not, however, discernible in the social interaction test (27). CGS 9896 was not a potent anticonvulsant when tested against high doses of PTZ (4) in rats, although we have shown it has anticonvulsant action in mice (36). CGS 9896 was an antagonist at some endpoints, as it antagonized the hyperphagic action of clonazepam (7,10), and antagonized the relaxant effects of diazepam (4). AHR 11797, a pyrroloquinazolone (Fig. 1), seems to be an unique BDZ receptor ligand because it was reported to be a selective muscle relaxant, but antagonized the anticonflict effects in rats (19) and monkeys (37) and the anticonvulsant effects of chlordiazepoxide in rats (19).

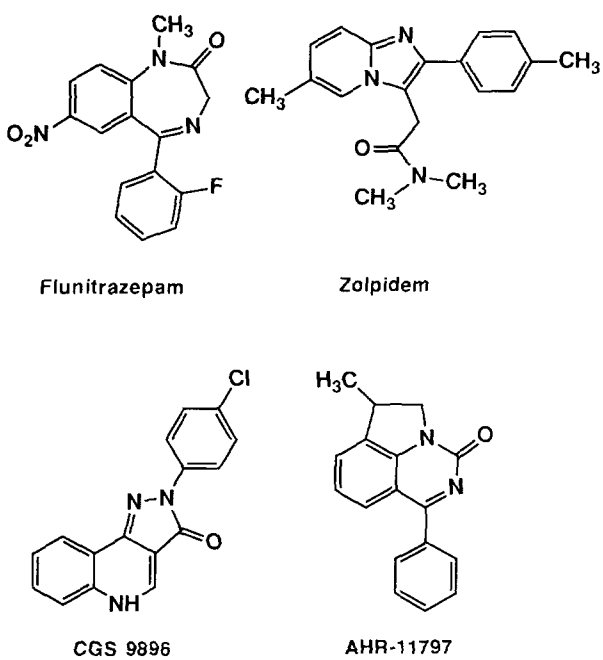

FIG. 1. Chemical structures of the four benzodiazepine receptor ligands studied.

METHOD

Animals

Male Long-Evans hooded rats weighing 250–500 g were used for the behavioral portion of this study (25). They were purchased from Harlan Sprague-Dawley (Indianapolis, IN) and Charles River (Wilmington, MA), and housed in groups of three or four. The animals were maintained on a reverse 12L : 12D cycle, with lights off at 0600 h. The rats were adapted to this reverse light : dark cycle for at least 2 weeks prior to drug treatment and behavioral testing. The animals were allowed free access to Purina rat chow (Diet 5012) and water at all times. On the test days, rats were transported from the dark holding room at 0900 h in a dark carrier to a room dimly illuminated with a 60 W red bulb. After 1 h of habituation to the new environment, drug or vehicle administration commenced, followed by behavioral testing.

Experimental Design

The experimental protocol for the behavioral studies was divided into three groups of tests. The first group of tests consisted of the measurements of changes in rectal temperature, performance in the elevated plus-maze, and locomotion in the open field, in the same group of animals. Food consumption following food deprivation was determined in a separate group of animals, as was anticonvulsant activity. Thirty minutes after intraperitoneal (IP) administration of vehicle or drug, in a volume of 1.0 ml/kg, the behavioral measurements commenced. For all Ro15-1788 antagonism experiments, Ro15-1788 was injected IP, 15 min after vehicle or drug injection.

Performance in the Automated Elevated Plus-Maze

The effects of these four compounds on the behavioral measures indicative of changes in anxiolytic- or anxiogenic-like profile were evaluated using a computer-controlled elevated plus-maze test system. The apparatus consisted of two open arms (50 × 10 cm) and two enclosed arms (50 × 40 × 10 cm) that were linked by a central platform (10 × 10 cm) and arranged such that the arms of the same type were opposite each other. The apparatus was constructed of perspex and mounted on a clear plastic base with a 50 cm elevation above the floor. The experimental procedures used were similar to those described by Pellow (27), according to the modification by Onaivi (24). The test system was further altered by the addition of 12 pairs of infrared photocell units. The photocells and their receivers were located 3 and 5 cm above the test platform at the entrances to each of the open and closed arms and also on the diagonal medians of the central platform. The interruption of the photocell beams by the animals was monitored via an interface (D-max 54, Newark) connected to

the IBM PC which was located in an adjacent room. With this arrangement, the movement and location of the rat during a 5-min test was continuously displayed, monitored, and recorded. The testing was initiated 30 min after vehicle or drug administration by placing each animal in the center of the plus-maze facing an open arm. The number of entries and the amount of time spent in the open arms, closed arms, and center platform were recorded. The period of time spent on the central platform was not included in the data analysis. The total time spent in the open and closed arms was usually less than 5 min. All measurements were performed with rats not previously used in any tests (16).

Measurement of Changes in Rectal Temperature

The animals were acclimatized to the laboratory conditions where the ambient temperature of the room was maintained at 23°C and humidity was about 40%. The rectal temperatures were recorded using a rat rectal probe inserted 6 cm into the rectum (Digital Thermometer, Fisher Scientific). Rectal temperatures were taken prior to the injection of vehicle or test compound and again 30 min after the treatment. The first reading was taken to familiarize the animals with the experimental procedure and ensure that drug responses were not masked by the small hyperthermic response that occurred when animals are initially handled (32). The rectal temperature of animals used in the Ro15-1788 antagonism experiments was taken every 15 min after the vehicle or drug injection for a period of 90 min.

Locomotor Activity Test

In this study, the observation of locomotion in an open field was adapted for the assessment of sedation and a general

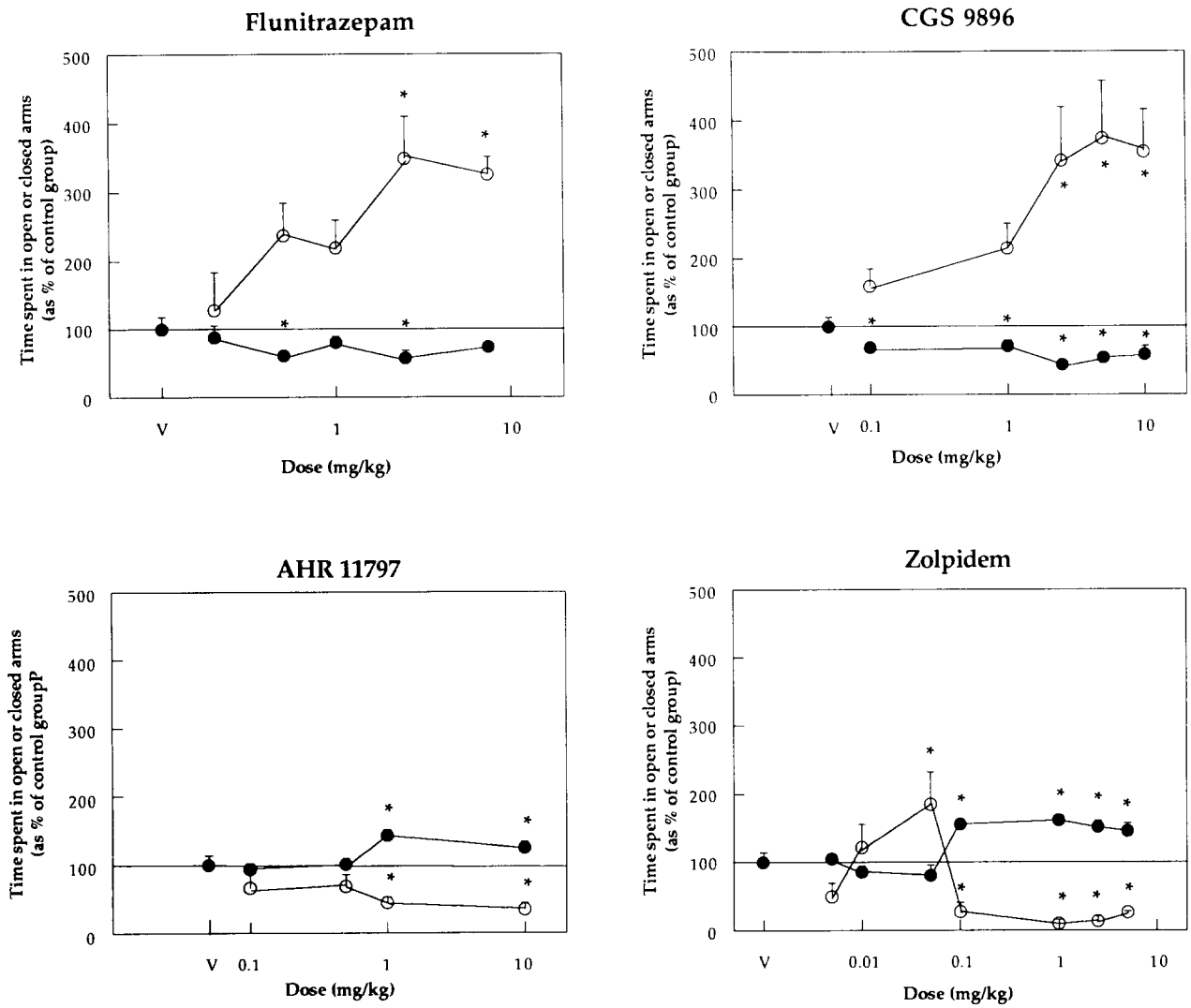

FIG. 2. Effect of the test compounds on performance in the elevated plus-maze. The parameter presented is the mean (± SEM, n = 5–10) amount of time spent by individuals in each particular dose group in the exposed arms of the maze. (○) Indicates time spent in the open arms and (●) indicate time spent in the closed arms. Values were normalized by expressing as percentage of control groups. Significant differences from controls ($p < 0.05$, calculated from a one-way ANOVA followed by Dunnett's t-test for multiple comparisons with vehicle-treated groups) are indicated with an asterisk.

depressant action following administration of vehicle or drug. The duration of observation was 10 min, begun immediately after evaluation in the elevated plus-maze. The locomotion activity apparatus consisted of circular test cages 46 cm in diameter and 34 cm high, each equipped with six pairs of photocells and detectors, equidistantly spaced, and placed 2 cm above the grid floor outside of the cage. Interruptions of the photocell beams were recorded by automatic digital counters.

Ingestional Behavioral Test

The palatable diet used in this experiment was modified rat chow pellets to which 15% sucrose had been added (Purina Mill, St. Louis, MO). A sham feeding experiment was conducted the day before the experiment day. Feeding behavior was assessed the next day following an additional 16 h period of food deprivation. On the test day, groups of rats were injected with doses of drug or vehicle and placed in individual cages. Thirty minutes later the test commenced with the introduction of 40 g chow pellets placed in a heavily weighted cup. The duration of the food consumption test was 1 h. At the termination of the test, the food remaining in was weighed and the amount consumed determined. During this test, water and the rat's standard chow were not available. We found this protocol to be sensitive to drug-induced increase or decrease in food consumption.

Anticonvulsant Test (PTZ-Induced Convulsions)

The ability of the test compound to prevent convulsions induced by PTZ (60 mg/kg IP) was tested by the intraperitoneal injection of the test compound 30 min before the injection of PTZ. The animals were then observed for a 30-min period for clonic and tonic convulsions. The number of subjects that convulsed and the duration of these convulsions were recorded. Rats that did not convulse after PTZ injection were assigned a 0 min duration, and lethal convulsions were not included in the data analysis.

Statistical Analysis

The behavioral results were analyzed using one-way analysis of variance (ANOVA) with multiple comparisons and the drug treatment as the independent factor. Dunnett's *t*-test was used to assess treatment differences.

Drugs

Flunitrazepam and Ro15-1788 were received as gifts from Hoffman-LaRoche (Nutley, NJ), CGS 9896 from Ciba-Geigy (Summit, NJ), zolpidem from Synthelabo, (Bagneux, France), and AHR 11797 from Wyeth Ayerst Research (Princeton, NJ). We synthesized additional AHR 11797 in our laboratory. Thin layer chromatography, ^{1}H-NMR and benzodiazepine receptor binding assays revealed no difference between the two samples of AHR 11797. For all tests, compounds were injected as a 40% w/v suspension of 2-hydoxypropyl β-cyclodextrin in deionized water purchased from Research Biochemical Inc. (Natick, MA).

RESULTS

Elevated Plus-Maze Test

Results from behavior observed in the elevated plus-maze are shown in Fig. 2. Of the four compounds studied, CGS 9896 and flunitrazepam exhibited anxiolytic profiles in this test paradigm, as evidenced by increased (over baseline levels) expenditure of time in the open arms [CGS 9896: $F(5, 35) = 4.355$, $p < 0.01$], [flunitrazepam: $F(5, 60) = 6.228$, $p = 0.0001$], and decreased time spent in the closed arms [CGS

TABLE 1

THE ABILITY OF Ro15-1788 TO ANTAGONIZE THE EFFECTS OF FLUNITRAZEPAM, AHR 11797, CGS 9896, AND ZOLPIDEM

Ro Dose (mg/kg)	Plus-Maze Test Time in Open Arms (% Control) 10	Rectal Temperature (°C) 10	Locomotion Activity (Interupts) 10	Food Consumption (g) 20	Anticonvulsant (% convulsed) 20
V + V	100 ± 13	39.2 ± 0.1	234 ± 13	2.6 ± 0.4	100
V + Ro	122 ± 17	39.3 ± 0.1	270 ± 22	2.6 ± 0.4	100
FLU dose (mg/kg)	1	7.5	7.5	2.5	5
FLU + V	224 ± 33*	37.1 ± 0.4*	104 ± 35*	6.7 ± 1.1*	0*
FLU + Ro	54 ± 22†	38.7 ± 0.3†	246 ± 25†	3.7 ± 0.4†	100†
AHR dose (mg/kg)	10	10	–	–	–
AHR + V	38 ± 8*	38.5 ± 0.2*	–	–	–
AHR + Ro	83 ± 11†	39.0 ± 0.1†	–	–	–
CGS dose (mg/kg)	10	10	10	5	–
CGS + V	180 ± 48*	38.7 ± 0.2*	178 ± 18*	4.9 ± 0.6*	–
CGS + Ro	63 ± 17†	39.4 ± 0.1†	252 ± 22†	3.5 ± 0.6†	–
ZOL dose (mg/kg)	5	7.5	7.5	–	–
ZOL + V	32 ± 10*	38.0 ± 0.1*	66 ± 19*	–	–
ZOL + Ro	94 ± 18†	39.2 ± 0.1†	226 ± 28†	–	–

*Different from vehicle (V)-treated group.

†Different from corresponding benzodiazepine-treated group. Significant difference ($p < 0.05$) were calculated by one-way ANOVA followed by Dunnett's *t*-test for multiple comparison with vehicle treated groups. Plotted values are Mean ± SEM (n = 6–10).

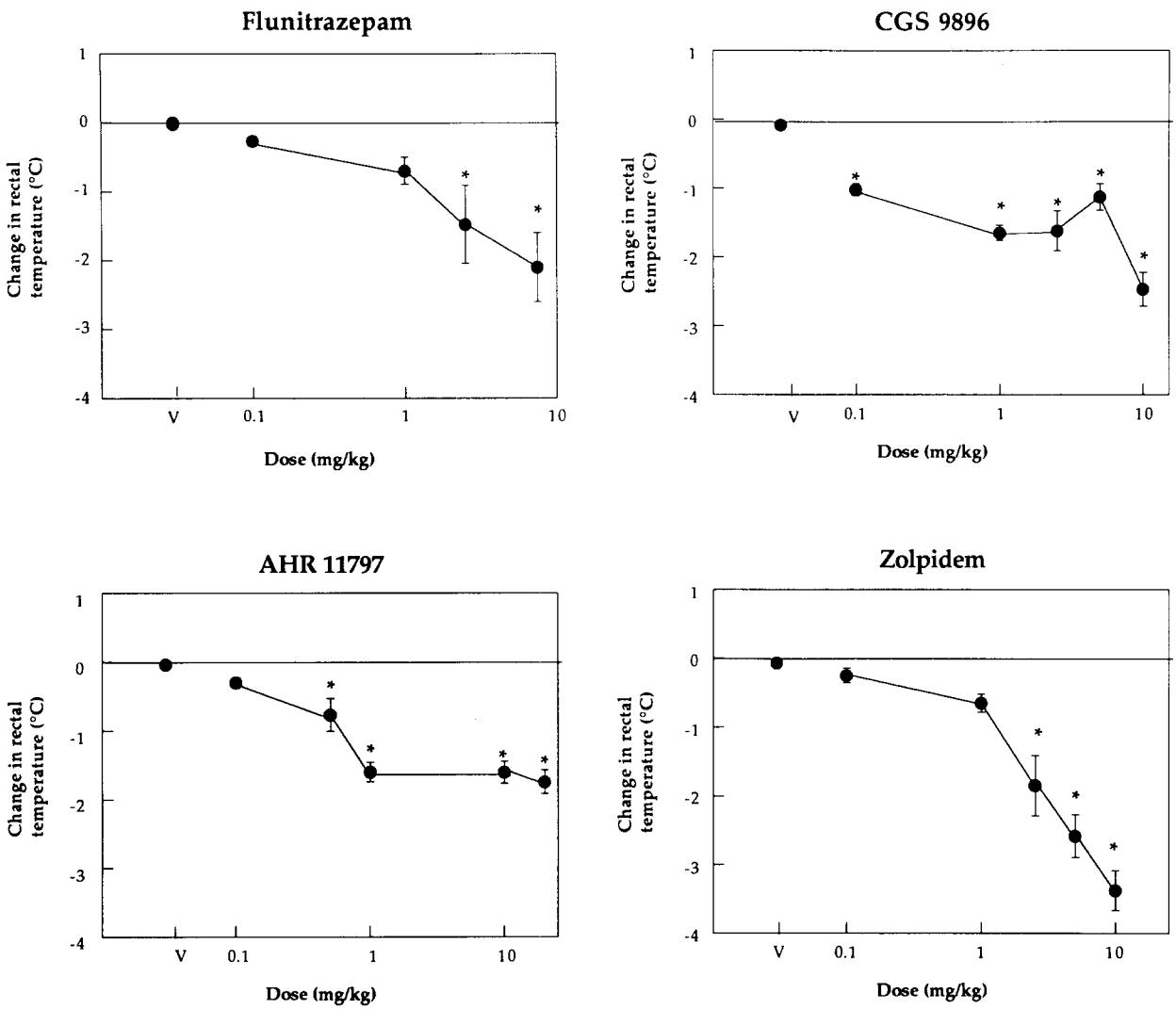

FIG. 3. The hypothermic effect of the four compounds. Plotted values are means ± SEM (n = 6–10). Significant differences from controls ($p < 0.05$, calculated from a one-way ANOVA followed by Dunnett's t-test for multiple comparisons with vehicle-treated groups) are indicated with an asterisk.

9896: $F(5, 35) = 7.302$, $p = 0.0001$] [flunitrazepam: $F(5, 60) = 2.98$, $p < 0.05$]. The proportion of entries into the open arms by these two compounds were also increased (not shown). By contrast, zolpidem showed a biphasic dose response curve, a dose of 0.05 mg/kg increased the time spent in the open arms ($t = 2.962$, $p < 0.05$) and at doses > 0.05 mg/kg decreased the time spent in the open arms, $F(7, 52) = 7.082$, $p = 0.0001$, and increased time in the closed arms of the plus-maze, $F(7, 52) = 13.66$, $p = 0.0001$. At all doses greater than 1 mg/kg, AHR 11797 reduced the time spent in open arms, $F(5, 57) = 5.069$ $p < 0.001$, and increased time in the closed arms, $F(5, 57) = 2.428$, $p < 0.05$. Ro15-1788, at 10 mg/kg, reversed the increase in time spent in the open arms caused by CGS 9896, $F(3, 30) = 3.645$, $p < 0.05$, and by flunitrazepam, $F(3, 30) = 9.898$, $p = 0.0001$. It also reversed the decrease in time spent in the open arms caused by AHR 11797, $F(3, 40) = 7.209$, $p = 0.0001$, and zolpidem, $F(3, 34) = 5.631$, $p < 0.05$ (Table 1).

Rectal Temperature

The changes in rectal temperatures following vehicle and drug administration are shown in Fig. 3. All four compounds produced qualitatively similar dose-dependent decreases of rectal temperature measurements. The maximal level of hypothermia produced by AHR 11797 was less than the maximal hypothermia produced by the other three compounds. The peak effect of Ro15-1788 on rectal temperature of all four drugs was found at 45 min postinjection of Ro15-1788 and 60 min after administration of drugs (data not shown). Table 1 shows that Ro15-1788 completely reversed the hypothermic effects of these compounds tested 60 min after administration.

Locomotor Activity in Open Field

As illustrated in Fig. 4, the locomotor activity test showed flunitrazepam, $F(3, 23) = 85.5$, $p = 0.0001$, and zolpidem, $F(8, 61) = 59.4$, $p = 0.0001$, to be markedly sedative while

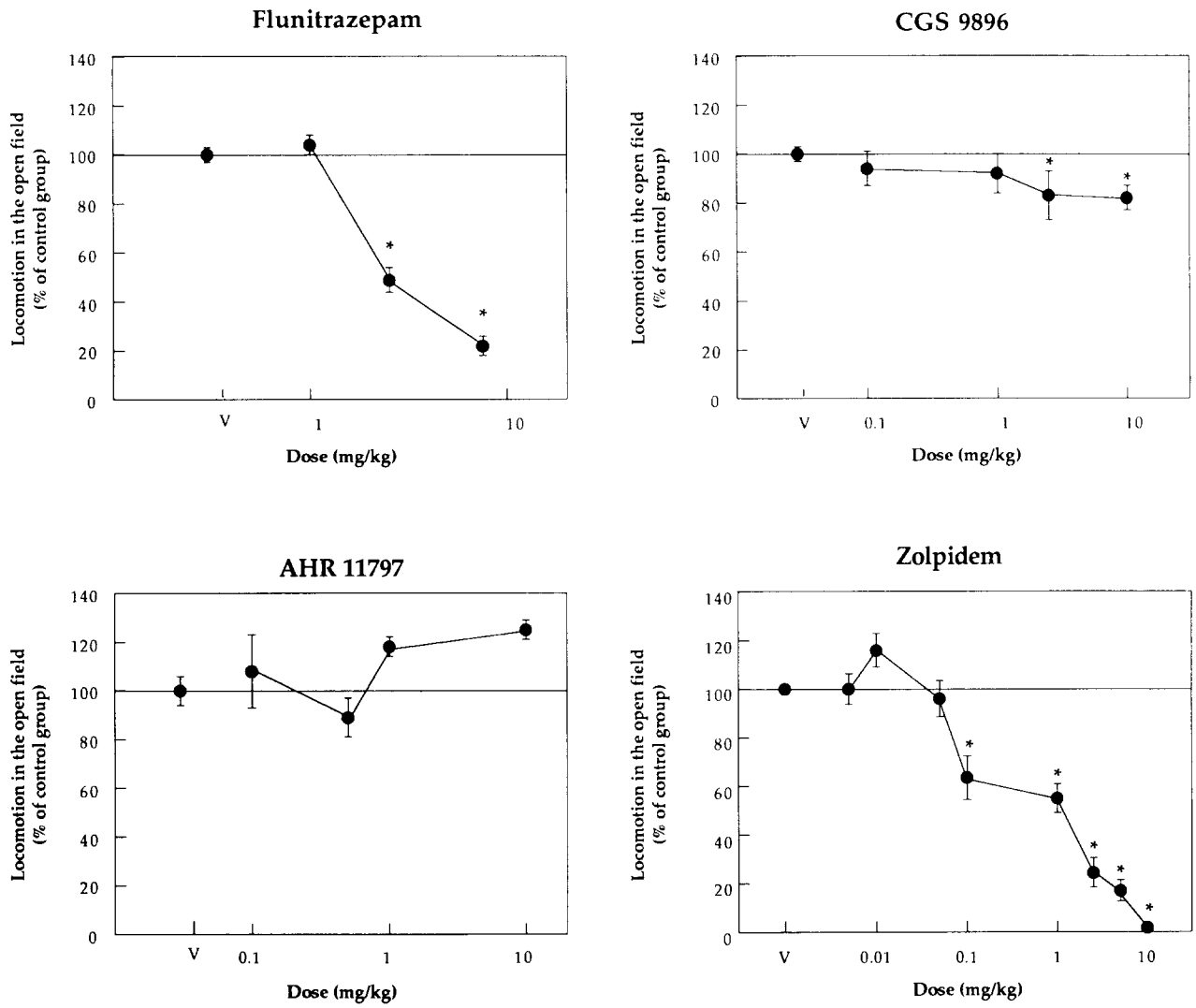

FIG. 4. The sedative effects of the four test compounds as reflected by levels of open-field activity. Plotted values are means (expressed as a percentage of control values) ± SEM (n = 6–10). Significant differences from controls ($p < 0.05$, calculated from a one-way ANOVA followed by Dunnett's t-test for multiple comparisons with vehicle-treated groups) are indicated with an asterisk.

CGS 9896 produced only a slight reduction in activity, $F(5, 39) = 1.346$, $p > 0.05$. AHR 11797 treatment, on the other hand, did not significantly affect locomotor activity. Ro15-1788 significantly antagonized the drug-induced depression of locomotion in flunitrazepam-, zolpidem-, and CGS 9896-treated rats (Table 1).

Food Consumption

The effects of these four compounds on amount of food consumed in 60 min by food-deprived rats are presented in Fig. 5. Flunitrazepam caused a dose-dependent increase of 200% in palatable food consumption, $F(5, 52) = 4.186$, $p = 0.0036$. AHR 11797 was not active at this endpoint. CGS 9896, $F(4, 40) = 8.981$, $p = 0.0001$, caused a significant dose-dependent increase in palatable food consumption. Even at doses that caused visible sedation, zolpidem had no effect on food consumption in this tested protocol. Ro15-1788 (20 mg/kg) completely reversed the hyperphagic effects by flunitrazepam and CGS 9896 (Table 1).

Anticonvulsant Activity

As shown in Fig. 6, of the four compounds studied, only flunitrazepam was able to fully protect the test animals from the convulsant effects of 60 mg/kg PTZ. AHR 11797 afforded no protection at all. Although zolpidem and CGS 9896 reduced the duration or severity, they did not prevent the convulsant event. We found that Ro15-1788 fully antagonized the protective effect of flunitrazepam as measured by proportion of animals that convulsed (Table 1).

DISCUSSION

The purpose of this study was to explore the possibility of behavioral selectivity as a consequence of functional multiple BDZ receptor subtypes. The qualitatively different effects and different rank order in the five in vivo endpoints by these four BDZ-like drugs, supports this hypothesis. Moreover, the ability of the central benzodiazepine receptor antagonist, Ro15-1788, to reverse all observed drug effects in the five in

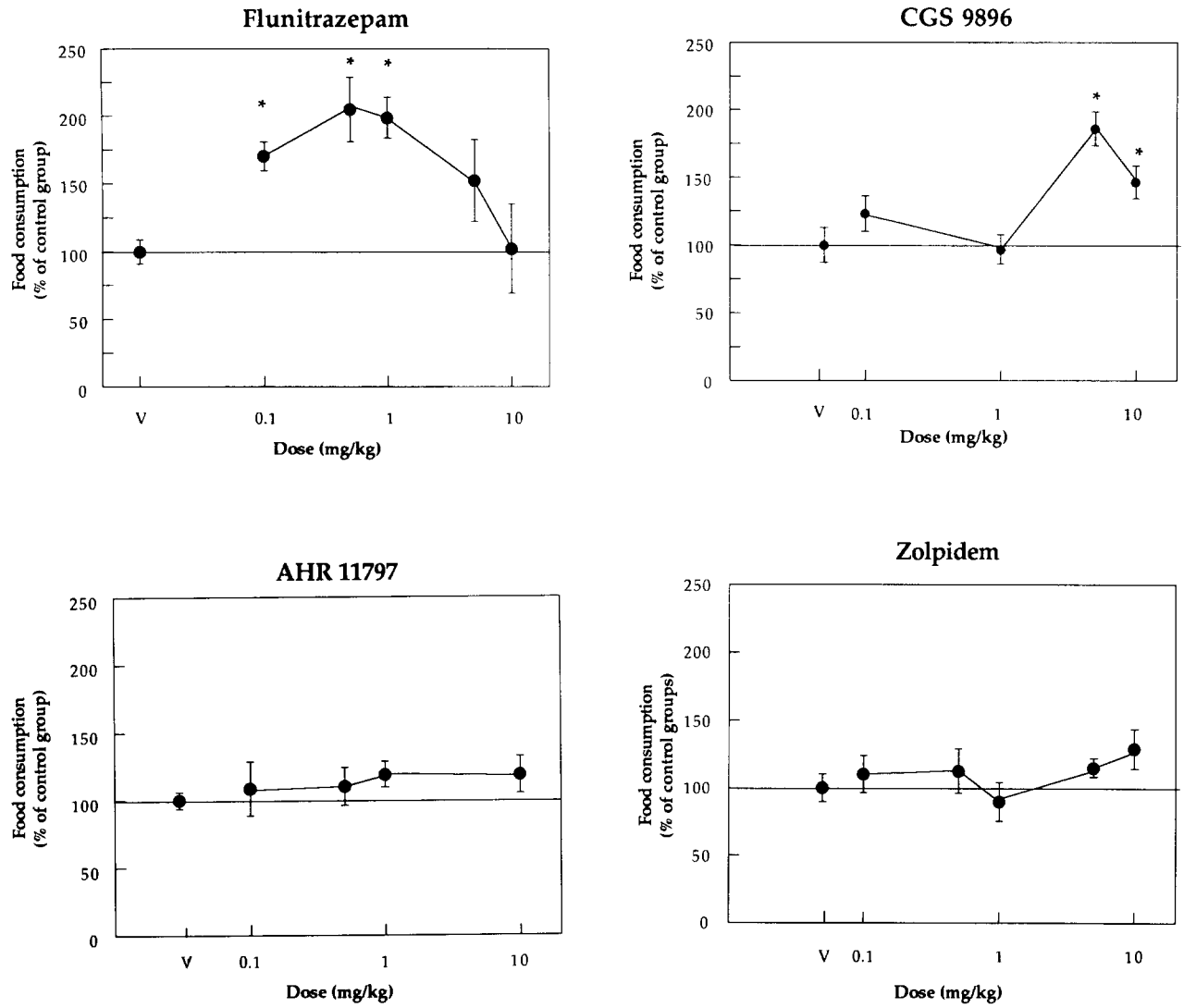

FIG. 5. Drug-induced changes in baseline levels of palatable food consumption following a period of food deprivation. Plotted values are mean amounts of food consumed by a given test group within the allotted period of time. Values are expressed as a percentage of control values ± SEM (n = 6–10). Significant differences from controls ($p < 0.05$, calculated from a one-way ANOVA followed by Dunnett's t-test for multiple comparisons with vehicle-treated groups are indicated with an asterisk.

vivo endpoints indicates that this receptor heterogeneity exists within the central benzodiazepine receptor population.

All four compounds decreased rectal temperature in a dose-dependent manner, and Ro15-1788 reversed this effect. This result indicates that they all bind to the subset of central benzodiazepine receptors that can affect body temperature. In contrast to Tang (34) who found Ro15-1788 only partially blocked the hypothermic effect of zolpidem in mice, we found Ro15-1788 fully blocked zolpidem-induced hypothermia in rats. AHR 11797 decreased body temperature, but this effect reached a plateau at a value less than the observed maximal hypothermia caused by the other drugs. This submaximal effect could suggest that AHR 11797 causes less than full activation of the BDZ receptor(s) responsible for causing hypothermia.

In the plus-maze test, these four compounds had qualitatively different effects. Flunitrazepam and CGS 9896 increased time spent in the open arms and decreased time spent in the closed arms; AHR 11797 had the opposite effect. Zolpidem displayed a biphasic dose response, causing an increase in the time spent in the open arms at a low dose and decreasing time in the open arms at higher doses that suppressed locomotor activity. Our results with the low doses of zolpidem (<0.15 mg/kg) agree with another plus-maze study (2) that showed zolpidem increased time spent in the open arms at low doses (<0.15 mg/kg) but did not have any effect at high doses (0.15 to 0.5 mg/kg). We have extended the dose range and found that doses above 0.1 mg/kg of zolpidem not only decreased time spent in the open arms, but, in spite of sedative effect of zolpidem, that rats also spent more time in the closed arms when compared to the control animals. The effect of zolpidem in the plus-maze is not consistent with anticonflict studies (14,23) that demonstrated an anticonflict action of zolpidem in punished drinking of thirsty rats. However, the use of the

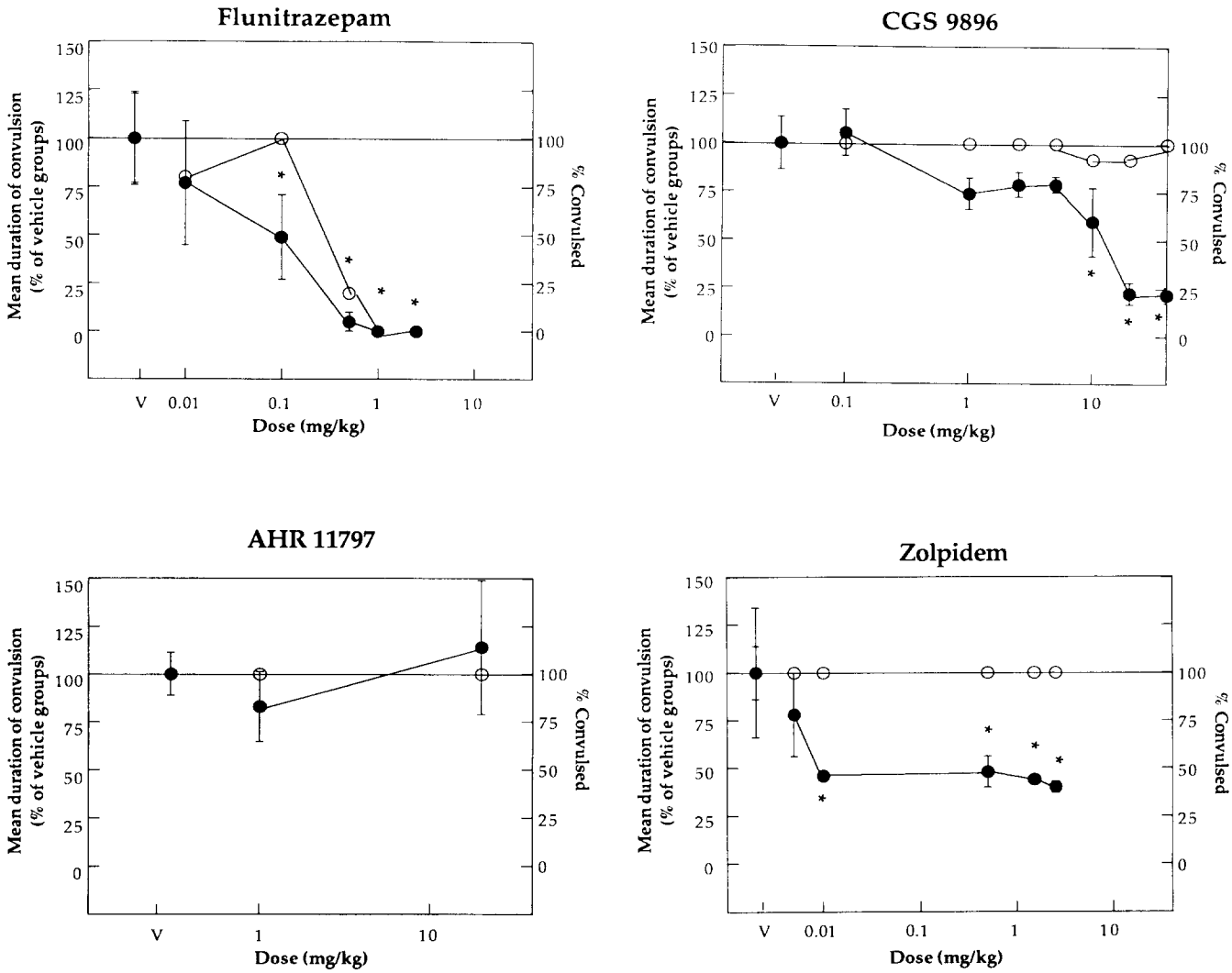

FIG. 6. Anticonvulsant properties of the four test compounds. The filled circles represent the mean duration of convulsions (clonic, tonic, or myclonic) occurring within the 30-min observation period. Values are expressed as a percentage of control values ± SEM (n = 5–12). Significant differences from controls ($p < 0.05$, calculated from a one-way ANOVA followed by Dunnett's t-test or multiple comparisons with vehicle-treated groups) are indicated with an asterisk. Open circles represent the number of animals within a dose group that convulsed, expressed as a percentage of the group population. Closed circles are the mean duration of convulsion.

punished drinking method to assess anxiolytic behavior is confounded by the ability of benzodiazepine ligands to directly affect water consumption (9). The anxiogenic profile of AHR 11797, as evidenced by the reduced amount of time spent in the open arms and increased amount of time spent in the closed arms of the plus-maze, is also a novel finding. Previous studies using a similar conflict test have indicated that AHR 11797 had no anticonflict activity and antagonized the anticonflict effects of diazepam (19). However, it is unlikely that the conditions used in this type of conflict test would have detected a proconflict effect indicative of an anxiogenic action because the level of responding in control animals was almost maximally suppressed by the punishment conditions.

This study has demonstrated that, at the doses tested, only zolpidem and flunitrazepam caused significant sedation, as assessed by decreased locomotor activity. In fact, both drugs have been used clinically as sedative/hypnotics. In agreement with other studies (4), a slight reduction in activity by CGS 9896 was also observed. This effect occurred at doses similar to that required for anxiolytic activity but significantly higher than was required to cause significant hypothermia. Unlike any other compound tested, AHR 11797 did not significantly affect activity at the highest dose tested. Those results indicated that CGS 9896 and AHR 11797 cause little or no activation of benzodiazepine receptor(s) responsible for suppression of locomotion.

Of the four compounds studied, only flunitrazepam could be classified as an effective anticonvulsant. In these tests, AHR 11797 did not increase or decrease the convulsant activity of PTZ; a result in agreement with a preliminary report that AHR 11797 had no effect and antagonized the anticonvulsant action of chlordiazepoxide (19). Zolpidem and CGS

9896 did reduce the severity of the convulsions but did not prevent them. It has been shown that the limited anticonvulsant action of CGS 9896 is most pronounced at lower doses of PTZ than used here (4). It is conceivable that the higher dose of PTZ and the route of administration used prevented CGS 9896 from protecting at the highest dose studied. However, there is other evidence that indicates that CGS 9896 is not an effective inhibitor of PTZ actions. Intracellular recording from cultured spinal cord neurons demonstrated that CGS 9896 alone had no effect on GABA responses (13) and did not alter the inhibition of GABA responses produced by PTZ, whereas diazepam antagonized this decrease (12). This result would suggest that the lack of anticonvulsant activity of CGS 9896 may be due to inability to substantially activate particular BDZ receptor subtypes that are responsible for that activity. Unfortunately, with the present state of knowledge of the BDZ receptor subtypes found on cultured spinal cord neurons, it is not possible to determine the identity of receptors that are minimally activated by CGS 9896.

In the feeding test, flunitrazepam dose dependently increased food consumption as is typical for 1,4 benzodiazepines (6). We also found that CGS 9896 produced a hyperphagic effect similar to flunitrazepam. These results differ from those obtained with the use of highly palatable diet (8) or saline and water intake (15) in which CGS 9896 has been shown to have no hyperphagic effect (7,10,11). The cause of the discrepancy could be due to differences in experimental protocols such as diet composition and duration of food deprivation. Because the basal amount of food consumed in our study was less than reported in other studies (7,10,11), it is possible that our protocol was able to detect a small increase of food consumption. It is interesting that in the plus-maze test, both CGS 9896 and flunitrazepam are equipotent, requiring 1 mg/kg to have a significant anxiolytic effect, while flunitrazepam is more active than CGS 9896 in feeding, leading to a significant increase at a much lower dose. This difference in rank order indicated that the BDZ receptor subtype(s) that mediate the anxiolytic and hyperphagic functions are not identical. We also found that neither AHR 11797 nor zolpidem affected food consumption. Our results for zolpidem are in agreement with those obtained in rats (10,39) and mice (28) in which it was found to have no effect on feeding.

The differential effects of AHR 11797, CGS 9896 and zolpidem, when compared to the effects of flunitrazepam, could be due to the differential ability to either activate or recognize diverse BDZ receptor subtypes. For example, it is possible that all four compounds, upon binding to receptor(s) involved in each endpoint, are potent agonists at some of them, weak agonist or antagonists at another population, and inverse agonists at still others. Alternatively, differences in the ability of these compounds to bind to BDZ receptor subtypes and increase the flow of Cl^- through the associated Cl^- channel could also account for the observed behavior. In such a scenario, two different neuronal pathways, acting in concert or in opposition to cause a specific end effect, might have different populations of BDZ receptors. The balance between these pathways may be uniquely shifted by each BDZ receptor ligand, thereby resulting in a qualitatively different behavioral profile.

The diversity of pharmacological profiles found for these four BDZ receptor ligands indicates that functional heterogeneity of central benzodiazepines exist and is a good indication that new compounds can be developed to have specific desired actions.

ACKNOWLEDGEMENTS

This work was supported by NIDA Grant #DA 06304-03 and is gratefully acknowledged. We thank Drs. Mark Beatty and Christopher Upton for synthesis of AHR 11797. We also wish to acknowledge collaboration in design of the automated plus-maze and consultation with the laboratory of Dr. George Koob and the helpful discussions with Dr. Mervyn Maze.

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